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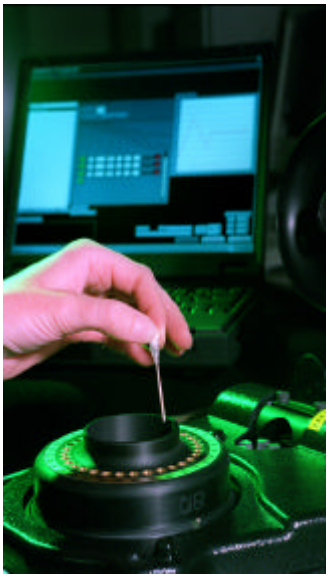
Detecting Pathogens with Real-Time PCR

Brucellosis in such wildlife species as bison and elk is endemic and is a concern for the cattle industry. INEEL researchers are developing a blood test to detect active *Brucella* infection in bison, other wildlife, and cattle. The detection of pathogens has traditionally been accomplished using a polymerase chain reaction (PCR) followed by electrophoretic gel separation of amplified target DNA. The approach typically requires about 3 hours for assay results. Our development of real-time PCR allows the amplification process to be monitored during each cycle of the PCR process, rather than following completion. This decreases the time required for detection to approximately 30 minutes and also enables quantification of starting target DNA. Probes and melt analysis of products can be used to increase specificity and determine the purity of a PCR product. We have acquired a field-portable real-time PCR instrument, called *RAPID*, for Ruggedized Advanced Pathogen Identification Device, and we are optimizing assays to detect *Brucella*. We are also developing and testing reliable primer sets for strain identification. These tools will greatly aid in the study to determine the true potential for interspecies transfer of *Brucella* in the environment.

Results

Yellowstone National Park has been the focus for our field tests, given the large number of bison and elk within its boundaries. Application of this new equipment and associated techniques will allow us to determine, on site, the infection of bison and elk. The information will aid in decisions regarding the need for destruction of animals. Furthermore, real-time PCR will allow the longstanding question of transfer between species to be addressed. In addition, a multiplex PCR/multiple probes assay holds the promise of allowing simultaneous detection and discrimination of several organisms in a sample.

In general, real-time PCR potentially applies to forensics and national security-related needs. Available field technology to meet law enforcement and international needs at border check points will aid in controlling the spread of pathogens and other unwanted materials.



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Selected Publications/Presentations

D.T. Newby, H.G. Silverman, T. Hadfield, and F. F. Roberto, "Real-time PCR Detection of *Brucella abortus*: a Comparative Study of SYBR Green I, Taqman, and Hybridization Probe Assays," American Society for Microbiology, 102nd General Meeting, Salt Lake City, UT, May 2002, Poster Q-381.

F. F. Roberto and D. T. Newby, "DNA Technology—An Introduction to Real-time PCR," Hispanic Youth Symposium (Scientific/Engineering Workshop), Sun Valley, Idaho, April 2001.

D. T. Newby and F.F. Roberto, "Application of the Ruggedized Advanced Pathogen Identification Device (RAPID) for *Brucella* detection," INEEL Biotechnology Seminar Series, July 2001.

F. F. Roberto, H. G. Silverman, M. Tsang, R. Rodriguez, "A PCR Diagnostic System for *Brucella* in Wildlife Species," USGS Bison Research Initiative, 1998–2000, Yellowstone National Park, Bozeman, Montana.